REMARKS

In view of the above amendments and the following remarks, reconsideration of the outstanding office action is respectfully requested.

Support for new claim 37 and the amendments to the claims is found, for example, in Figures 1 and 5 (and their accompanying description on page 8, lines 5-11, page 8, line 30 to page 9, line 5, and page 18, lines 17-25), page 48, lines 20-23, and the Examples of the Specification.

The rejection of claim 1, and claims 2-17 dependent thereon, under 35 U.S.C. § 112, second paragraph, for indefiniteness is respectfully traversed in view of the above amendments.

The rejection of claims 1-4, 7, and 9 under 35 U.S.C. § 102(b) as anticipated by Wu et al., "Production of Transgenic Rice Plants that are Resistant to Insect Pests and Fungal Diseases or to Water and Salt Stress," General Meeting of the International Program on Rice Technology, abstract 113 (1997) ("Wu I") is respectfully traversed.

Wu I is an abstract that discloses using constitutive or ABA-inducible promoters to drive water stress or salt stress tolerance genes in transgenic rice plants.

It is the position of the U.S. Patent and Trademark Office ("PTO") that Wu I teaches the production of water stress or salt stress tolerant transgenic rice plants and the use of constitutive or ABA-inducible promoters. Although Wu I does not explicitly teach an ABRC unit, the PTO argues that an ABA-inducible promoter would inherently comprise at least one abscisic acid response complex ("ABRC") unit.

Wu I neither discloses nor suggests an expression cassette comprising at least one ABRC unit, a minimal promoter necessary and sufficient for promoter activity, and a DNA molecule that increases tolerance to salt stress and drought stress in plants, as required by the claims of the present application. In particular, the claims of the present invention require a composite inducible promoter which includes at least one ABRC unit and a minimal promoter necessary and sufficient for promoter activity. Wu I merely discloses an ABA-inducible promoter.

As set forth in the Declaration of Ray J. Wu Under 37 C.F.R. § 1.132 ("Wu Declaration") filed herewith, Wu I does not teach or suggest an ABRC composite promoter, which includes at least one ABRC unit and a minimal promoter necessary and sufficient for promoter activity linked together to permit expression of a DNA molecule in the leaves or roots of a plant, as claimed in the present application. In particular, the disclosure of an

ABA-inducible promoter in Wu I would not necessarily teach or suggest a composite promoter, as claimed in the present application (Wu Declaration ¶ 6). For example, numerous non-composite, ABA-inducible promoters exist in nature (Wu Declaration ¶ 6). However, a naturally occurring ABA-inducible promoter does not necessarily function in the same way as an ABRC composite promoter, as claimed in the present application (Wu Declaration ¶ 6). In particular, a naturally occurring ABA-inducible promoter, such as the Hva22 promoter from barley, is a seed specific ABA-inducible promoter (Wu Declaration ¶ 6). Thus, this naturally occurring ABA-inducible promoter is not suitable for driving the expression of a foreign gene to confer stress tolerance to a plant (Wu Declaration ¶ 6). This is because, in order to make a plant tolerant to abiotic stresses, the DNA molecule that increases tolerance to salt stress and drought stress needs to be expressed in the leaves and roots of the plant (Wu Declaration ¶ 6).

In contrast, a composite promoter including an ABRC unit and minimal promoter necessary and sufficient for promoter activity, as claimed in the present application, functions in the leaves and/or roots of a transgenic plant (Wu Declaration ¶ 7). Thus, the inserted DNA molecule that increases tolerance to salt stress and drought stress in plants can be expressed in the leaves and/or roots of the transgenic plant to protect the plant from these abiotic stresses in accordance with the present invention (Wu Declaration ¶ 7).

As Wu I merely discloses an "ABA-inducible" promoter, it does not teach or suggest an ABRC composite promoter including at least one ABRC unit and a minimal promoter necessary and sufficient for promoter activity which can be used to express an inserted DNA molecule in the leaves and/or roots of a plant, as required by claims 1-17 and 37 of the present application.

Moreover, as described above, the mere disclosure of an "ABA-inducible" promoter is not sufficient to enable one of ordinary skill in the art to confer tolerance to salt stress and drought stress in a monocotyledonous plant, as claimed in the present application. Accordingly, the rejection based on Wu I is improper and should be withdrawn.

The rejection of claims 1-17 under 35 U.S.C. § 103(a) as being unpatentable over Wu I in view of Applicants' admitted prior art is respectfully traversed in view of the above remarks.

The rejection of claims 1-17 under 35 U.S.C. § 103(a) as being unpatentable over Xu et al., "Expression of a Late Embryogenesis Abundant Protein Gene, *HVA1*, from Barley Confers Tolerance to Water Deficit and Salt Stress in Transgenic Rice," Plant Physiol., 110:249-257 (1996) ("Xu") in view of Shen et al., "Modular Nature of Abscisic

Acid (ABA) Response Complexes: Composite Promoter Units That Are Necessary and Sufficient for ABA Induction of Gene Expression in Barley," The Plant Cell, 8:1107-1119 (1996) ("Shen"), and further in view of Applicant's admitted prior art is respectfully traversed.

Xu discloses introducing the *HVA1* gene from barley into rice cells using the biolistic-mediated transformation method to generate transgenic rice plants. Xu teaches that expression of the *HVA1* gene regulated by the rice actin 1 gene promoter led to high-level, constitutive accumulation of the HVA1 protein in the transgenic rice plants which exhibited significantly increased tolerance to water deficit and salt stress.

Shen discloses the sequence for an abscisic acid response complex ("ABRC") from a barley *HVA1* gene. Shen teaches that the combination of different ACGT-boxes and coupling elements leads to the formation of ABRCs with different transcription strengths in a transient assay. This reference also suggests that the disclosed synthetic promoters (including an ABRC and the Amy64 minimal promoter) capable of conferring different levels of ABA induction could be used to drive the expression of genes that would enhance plant stress tolerance.

It is the PTO's position that it would have been obvious to a person of ordinary skill in the art to use a minimal promoter operably linked to an ABRC, as taught by Shen, to express a DNA molecule that increases tolerance to salt stress or drought stress in plants, as taught by Xu, for the purpose of conferring salt stress or drought stress tolerance to a monocot plant. Applicants respectfully disagree.

A proper *prima facie* showing of obviousness requires the PTO to satisfy three requirements. First, the prior art relied upon, coupled with knowledge generally available to one of ordinary skill in the art, must contain some suggestion which would have motivated the skilled artisan to combine or modify references. See In re Fine, 837 F.2d 1071, 1074, 5 USPQ2d 1596, 1598 (Fed. Cir. 1988). Second, the PTO must show that, at the time the invention was made, the proposed modification had a reasonable expectation of success. See Amgen v. Chugai Pharm. Co., 927 F.2d 1200, 1209, 18 USPQ2d 1016, 1023 (Fed. Cir. 1991). Finally, the combination of references must teach or suggest each and every limitation of the claimed invention. See In re Wilson, 424 F.2d 1382, 1385, 165 USPQ 494, 496 (CCPA 1970).

Applicants maintain that the PTO has failed to show any reasonable expectation of success. Merely offering a scientist an opportunity to do many months of experimentation is not sufficient to show obviousness. Although Shen suggests that the

disclosed synthetic promoters capable of conferring different levels of ABA induction could be used to drive the expression of genes that would enhance plant stress tolerance, that reference provides no reasonable expectation of success for a method for conferring tolerance to salt stress and drought stress in a monocot plant comprising transforming the monocot plant with an expression cassette comprising at least one ABRC unit, a minimal promoter, and a DNA molecule that increases tolerance to salt stress and drought stress in plants, as required by the claims of the present application. In particular, a showing of ABA-induction alone for a particular composite promoter in a transient assay, as in Shen, does not teach or suggest conferring salt or water stress tolerance in transgenic plants, since not all ABA-inducible promoters are suitable for driving the expression of a foreign gene to confer stress tolerance to a plant (Wu Declaration ¶ 11). Moreover, as set forth in the Wu Declaration, one of ordinary skill in the art would not be able to determine from the disclosures of Xu and Shen whether a minimal promoter linked to an ABRC unit would be able to confer tolerance to water or salt stress in transgenic plants, let alone achieve results at least equivalent to those of the constitutive promoter of Xu, which is identified in Xu as producing transgenic plants showing "significantly increased tolerance to water deficit and salinity." In particular, it was necessary for the present inventors to experimentally determine whether a minimal promoter linked to an ABRC unit, as claimed in the present application, would confer suitable tolerance to water or salt stress in transgenic plants (Wu Declaration ¶ 11).

As set forth in the present application and in the Wu Declaration, the present inventors have unexpectedly determined that transgenic plants transformed with a DNA molecule that increases tolerance to salt stress and drought stress in plants driven with a composite promoter of the present invention, including at least one ABRC unit and a minimal promoter, exhibit improved tolerance to salt stress and drought stress as compared to transgenic plants transformed with a DNA molecule that increases tolerance to salt stress and drought stress in plants driven with a constitutive promoter (Wu Declaration ¶ 12).

In particular, the present inventors performed multiple parallel experiments using a constitutive promoter and a composite promoter of the present invention including at least one ABRC unit and a minimal promoter (Wu Declaration ¶ 13). One set of experiments is described in Examples 11 and 12 of the above-identified application (see Specification at page 26, line 39 to page 28, line 31 and page 18, line 5 to page 26, line 37 for a detailed description of production of plasmids, transgenic plants, and exposure to water or salt stress conditions) (Wu Declaration ¶ 13). In particular, transgenic plants including a constitutive promoter (rice line JS102) and transgenic plants including a composite promoter of the

present invention (which includes four ABRC units and a minimal promoter necessary and sufficient for promoter activity -- Act1-100) (rice lines JS112 and JS110) were subjected to water stress and salt stress conditions (Wu Declaration ¶ 13). As shown in Table 3 (see Specification at page 27, lines 19-41), under water stress (top half of Table 3) or salt stress (bottom half of Table 3) conditions, transgenic rice plants that used a composite promoter of the present invention to drive the expression of the Δ^1-pyrroline-5-carboxylate synthetase gene (rice line JS112) grew much faster as compared to plants that used a constitutive promoter (rice line JS102) (Wu Declaration ¶ 13).

Moreover, a second set of experiments was conducted, in which it was unexpectedly determined that transgenic plants transformed with a DNA molecule that increases tolerance to salt stress and drought stress in plants driven with a composite promoter including at least one ABRC unit and a minimal promoter exhibit improved tolerance to salt stress and drought stress as compared to transgenic plants transformed with a DNA molecule that increases tolerance to salt stress and drought stress in plants driven with a constitutive promoter (Wu Declaration ¶ 14). In particular, when plants are being stressed by high salinity or drought conditions, the cell membranes become leaky and ionic compounds leak out of the cells (Wu Declaration ¶ 14). Extensive leakage of ions and other cellular contents will lead to the death of cells and tissues (Wu Declaration ¶ 14). Therefore, the extent of leakage of ions is a measure of the health of the plant. The higher extent of leakage, the less healthy the plant (Wu Declaration ¶ 14). A common method for measuring the extent of leakage of ions is to determine the electrical conductivity of the water used to soak the leaves (Wu Declaration ¶ 14). This method was applied as described below (Wu Declaration ¶ 14).

Transgenic plants including a constitutive promoter (rice *Act1* promoter) and the *Hva1* gene (pRKJ6) were prepared, as described in Rohila et al., "Genetic Improvements of Basmati Rice for Salt and Drought Tolerance by Regulated Expression of a Barley *Hva1* cDNA," Plant Sci. 163:525-532 (2002) ("Rohila") (copy attached as Exhibit A to the Wu Declaration) (Wu Declaration ¶ 15). In addition, transgenic plants including a composite promoter of the present invention (which includes four ABRC units and a minimal promoter necessary and sufficient for promoter activity -- Act1-100) and the *Hva1* gene (pRKJ21) were prepared, as described in Rohila (Wu Declaration ¶ 15). Briefly, three-week old calli of rice cv. Pusa Basmati 1 were co-cultivated with *Agrobacterium* strain LBA4404 harboring pRKJ6 or pRKJ21 (Wu Declaration ¶ 15). Selection of transformed calli and regeneration of

plantlets were carried out as described in Rohila (Wu Declaration ¶ 15). The transgenic plants were then subjected to salt stress conditions and electrical conductivity of the resulting plants was measured (Wu Declaration ¶ 15). In particular, mature seeds harboring a single copy of the transgene were selected from independent transgenic R2 rice lines from each plasmid (i.e., RKJ6 and RKJ21) (Wu Declaration ¶ 15). These seeds, as well as seeds from non-transgenic and untransformed controls, were sown on Petri dishes lined with moist filter paper (Wu Declaration ¶ 15). Later on, the plants were grown for three weeks in soil in the greenhouse (Wu Declaration ¶ 15). For determination of salt stress, three-week-old plants were irrigated daily with 200 mM saline water to maintain the salinity level (Wu Declaration ¶ 15). After eight days of stress, salt was flushed out by heavy irrigation of pots with tap water twice a day for two days (Wu Declaration ¶ 15). Then, two additional cycles of salt stress were carried out (Wu Declaration ¶ 15). After completion of the salt stress, the next leaf to the flag leaf was removed and cut into small pieces (Wu Declaration ¶ 15). The pieces of leaves were immediately put into a test tube containing 2.5 ml cold water, and gently vortexed for 15 seconds (Wu Declaration ¶ 15). The tubes were placed in a dessicator, and a vacuum was applied for five minutes to remove air bubbles from the surface of leaf tissues (Wu Declaration ¶ 15). The tubes were then covered and placed at room temperature for 24 hours (Wu Declaration ¶ 15). A VWR conductivity meter with a platinum electrode and built-in temperature correction was used to measure the electrical conductivity of the solution (Wu Declaration ¶ 15). The results are shown in Table 1, below (Wu Declaration ¶ 15).

Table 1. Electrical conductivity readings from R2 plant leaves after salt stress

Plasmid	Line #	Electrical conductivity (μmho/mg leaf)			
		Average value[a] per plant line	Average of 4 transgenic lines	%[b]	Average % of 4 transgenic lines
pRKJ6 (*Act1-Hva1)*	42	3600 ± 60		63	
	44	3830 ± 186	4020	67	70.0
	220	4540 ± 138		79	
	289	4110 ± 107		71	
	Neg[c]	5755 ± 65		100	
	NT[d]	5850 ± 35		102	
pRKJ21 *(4 ABRC-Hva2)*	12	3160 ± 80		55	
	13	3360 ± 78	3435	58	59.5
	14	3570 ± 40		62	
	96	3650 ± 120		63	
	Neg[c]	5795 ± 145		100	
	NT[d]	5920 ± 300		102	

[a]The values are mean from three replicates in each line.
[b]Indicates the percentage of the electrical conductivity of each transgenic plant line and NT over that of Neg control plants, which is set as 100%.
[c]Neg is non-expressing, transgenic line as a control.
[d]NT is untransformed control.

As shown in Table 1, *Hva1*-producing transgenic plants driven by the composite promoter of the present invention were healthier, as reflected by lower extents of ion leakage from leaves under salt stress, compared to those plants with *Hva1* driven by a constitutive promoter (Act1 promoter) (Wu Declaration ¶ 16).

In a third set of experiments, production of glycine betaine in transgenic rice plants harboring the choline oxidase gene (*Cox*) from *Arthrobacter pascens* was tested (Wu Declaration ¶ 17). Transgenic plants where *Cox* was driven by a composite ABRC-inducible promoter of the present invention (including four ABRC units and a minimal promoter necessary and sufficient for promoter activity -- Act1-100 (identical to pJS112, except the P5CS cDNA is replaced by the Cox cDNA)) and transgenic plants where *Cox* was driven by a constitutive promoter (ubiquitin promoter) were produced using the same general methodology as described the Examples of the above-identified patent application (Wu Declaration ¶ 17). 24-day old rice plants were stressed with 150 mM NaCl for six days, watered for seven days, and then stressed with 150 mM NaCl for 12 days (Wu Declaration ¶ 17). Plant biomass was measured after watering for ten days (Wu Declaration ¶ 17). The results are shown in Table 2, below (Wu Declaration ¶ 17).

Table 2
Salt stress tolerance test of *Cox* transgenic rice plants.*

Plants	Promoter Used to Drive *Cox*	Fresh Root Weight[§]	Fresh Shoot Weight[§]
Non-transgenic	None	0.21	2.2
		0.24	2.4
Transgenic	Inducible (ABRC)	0.9	6.0
		1.2	6.8
		1.3	7.7
Transgenic	Constitutive (ubiquitin)	0.4	3.2
		0.5	3.7
		0.6	3.7

**Cox* is the choline oxidase gene from *Arthrobacter pascens*.
[§]Gram per plant. Average of 6 plants per line.

As shown in Table 2, transgenic rice plants grew at least twice as fast after salt stress when the *Cox* gene was driven by an ABRC-inducible composite promoter of the present invention, as compared to plants where the *Cox* gene was driven by a constitutive promoter (Wu Declaration ¶ 18).

Accordingly, in all three sets of parallel experiments using a constitutive promoter and a composite promoter of the present invention, the composite promoter of the present invention unexpectedly resulted in improved tolerance of transgenic plants to salt stress and drought stress (Wu Declaration ¶ 19).

As Shen provides no experimental evidence or guidance regarding whether an ABRC unit linked to a minimal promoter would confer suitable tolerance to water or salt stress in transgenic plants, the rejection based on Xu and Shen is improper and should be withdrawn.

Claims 1-17 are provisionally rejected under 35 U.S.C. § 103(a) for obviousness over copending U.S. Patent Application Serial No. 09/107,201 to Wu et al. ("'201 application"). This rejection is obviated in view of the abandonment of the '201 application.

Claims 1-17 are provisionally rejected under 35 U.S.C. § 103(a) for obviousness over copending U.S. Patent Application Serial No. 09/339,364 to Wu et al.

("'364 application"). This rejection is obviated in view of the abandonment of the '364 application.

The rejection of claims 1-17 under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-7 and 10 of U.S. Patent No. 5,981,842 to Wu et al. ("Wu II") is respectfully traversed.

Claims 1-7 and 10 of Wu relate to a method of producing a cereal plant cell or protoplast for regeneration of a water stress or salt stress tolerant cereal plant by transforming a cereal plant cell or protoplast with a nucleic acid encoding a group 3 late embryogenesis abundant protein and a method of increasing tolerance of a cereal plant to water stress or salt stress conditions by increasing levels of a late embryogenesis abundant protein by transforming the plant with a nucleic acid encoding a group 3 late embryogenesis abundant protein.

It is the PTO's position that the use of ABRC units, minimal promoters, and *Agrobacterium*-mediated transformation in the methods of the instant invention would have been an obvious optimization of design parameters, because ABRC units, minimal promoters, and the use of *Agrobacterium*-mediated transformation were known in the art at the time of Applicants' invention. Accordingly, the PTO argues that claims 1-17 of the present application are not patentably distinct from claims 1-7 and 10 of Wu II. Applicants respectfully disagree.

In particular, claims 1-7 and 10 of Wu II neither disclose nor suggest "transforming the monocotyledonous plant with an expression cassette comprising at least one abscisic acid response complex unit, a minimal promoter necessary and sufficient for promoter activity, and a DNA molecule that increases tolerance to salt stress and drought stress in plants . . . operably linked together to permit expression of the DNA molecule in the leaves or roots of the plant," as required by the claims of the present application, as amended. As described above, a showing of ABA-induction alone for a particular promoter or composite promoter, as in Shen, does not teach or suggest conferring salt or water stress tolerance in transgenic plants, since not all ABA-inducible promoters are suitable for driving the expression of a foreign gene to confer stress tolerance to a plant (Wu Declaration ¶ 11). Moreover, it was necessary for the present inventors to experimentally determine whether a minimal promoter linked to an ABRC unit, as claimed in the present application, would confer suitable tolerance to water or salt stress in transgenic plants, as described above. Accordingly, this rejection is improper and should be withdrawn.

Claims 1-17 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 3-5, 11-12, 15, 46-55, and 72 of the '201 application. This rejection is obviated in view of the abandonment of the '201 application.

Claims 1-17 are provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-30 of the '364 application. This rejection is obviated in view of the abandonment of the '364 application.

Applicants note that the outstanding office action in the above-identified application was incorrectly sent to the wrong address. Accordingly, enclosed herewith is a copy of the Correction of Correspondence Address filed on February 14, 2002.

In view of the all of the foregoing, applicants submit that this case is in condition for allowance and such allowance is earnestly solicited.

Respectfully submitted,

Date: February 27, 2004

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